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Original Communication

The protective effect of chrysin against carbon tetrachloride-induced kidney and liver tissue damage in rats

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Abstract: The aim of this study was to investigate the possible protective effects of chrysin on oxidative status and histological alterations against carbon tetrachloride (CCl_4)-induced liver and kidney tissue in rats. The animals were randomly divided into four groups; the control, chrysin (100 mg/kg), CCl_4 (0.5 ml/kg) and chrysin + CCl_4 groups. Liver and kidney injuries were assessed by biochemical and histopathological examinations. The levels of malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD) activity were measured in tissues. Serum tumor necrosis factor-α (TNF-α), aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, and creatinine levels were also measured in blood samples. MDA, serum TNF-α, AST, ALT, urea, and creatinine levels ($p < 0.05$) were significantly higher, and SOD activity and GSH level were significantly ($p < 0.05$) lower in the CCl_4 group than in the control group. Treatment with chrysin in the chrysin + CCl_4 group decreased MDA, AST, ALT, creatinine, and TNF-α levels ($p < 0.05$), and increased SOD activity, GSH levels ($p < 0.05$), and serum TNF-α levels ($p < 0.05$). In addition, body weight change (BWC) ($p < 0.05$) and feed intake (FI) were significantly lower ($p < 0.001$) in the CCl_4 group than in the control group. Moreover, treatment with chrysin increased BWC and FI in the chrysin + CCl_4 group compared with that in the CCl_4 group. These findings also confirmed by histopathological examination. The chrysin treatment ameliorated the CCl_4-induced biochemical and pathological alterations. These results demonstrated that chrysin provided amelioration on the rat liver and kidney tissues CCl_4-induced injury by increasing the antioxidant activity.

Keywords: Chrysin, carbon tetrachloride, antioxidant, kidney, liver

Introduction

Carbon tetrachloride (CCl_4) is the most commonly used agent because it induces injuries similar to the development of cirrhosis in humans, although liver damage can be caused by many other factors in experimental studies [1]. This agent is found in high levels in several tissues, including the liver, brain, kidney, muscle, lung and testes, and is distributed throughout the body [2, 3]. CCl_4 that enters the body undergoes biotransformation in the liver and turns into a trichloromethyl (CCl_3) radical. It then rapidly combines with oxygen to form the trichloromethyl peroxide (CCl_3O_2) radical or chloroform form. The CCl_3O_2 radical is a known powerful lipid peroxidation initiator that damages the cell membrane and leads to oxidative damage by causing CCl_4 lipid peroxidation. Liver cells and fibroblasts are stimulated to produce extracellular matrix and synthesize collagen under oxidative damage conditions [4]. In addition, Kupffer cells, which are stimulated by damage, cause the production of proinflammatory cytokines, tumor necrosis factor-α (TNF-α), and interleukin-1 h (IL-1 h). Therefore, inhibition of oxidative stress could provide positive results [5]. Experimental studies have shown that CCl_4 causes liver degeneration, mononuclear cell infiltration, fibrosis, cirrhosis, and cancer [6, 7]. In recent years, compounds isolated from plants have become popular because they are natural, cheaper, easier to access, less toxic, and produce fewer adverse-effects [8]. Phytochemicals are compounds that are capable of

scavenging free radicals and inhibiting oxidative stress. Flavonoids, which have strong antioxidant properties, are abundant and widely available in plant. Chrysin is a recently studied flavonoid. The plant called Passiflora caerulea contains chrysin, as well as in propolis, celery, thyme, and honey [9]. It is used frequently in herbal treatments in China and East Asia. Flavanoids confer their antioxidant effects by chelating trace elements or radicals [10, 11]. According to the results of previous studies, chrysin exhibits many pharmacological effects due to its antioxidant, anticancer, anti-inflammatory activities [9, 12]. It is thought that chrysin can neutralize carcinogenic substances by reducing the level of free radicals in the cells, thereby contributing to the toxic effects on cancer cells and preventing tumor formation [13, 14, 15].

The aim of this study was to determine the effects of chrysin, which is known to have antioxidant and anti-inflammatory activities, on feed intake (FI), body weight change, lipid peroxidation, the antioxidant system, inflammation parameters, and histopathological changes, and apoptosis in rats with CCl_4-induced liver and kidney damage.

Materials and methods

Chemicals

CCl_4 (99.9%, Panreac Quimica, Barcelona, Spain) was dissolved in corn oil (5 ml/kg). Chrysin (100 g, Molekula Ltd., Wimborne Dorset, UK) was also dissolved in corn oil. All other chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo, USA).

Animals

Twenty-four adult Sprague Dawley male rats (6–8 weeks old, 280–300 g) were used in this study. The animals were obtained from the Firat University Experimental Research Center, Elazig, Turkey and were housed under standard laboratory conditions (temperature 24 ± 3 °C, humidity 40–60%, and a 12 h light:dark cycle). The rats were fed with a balanced commercial diet (Elazig Food Company, Elazig, Turkey), and had access to fresh drinking water *ad libitum*. The experiment was approved by the Animal Experiments Local Ethics Committee (10.02.2015/No:17).

Experimental Design

In this study, the rats were randomly divided into 4 groups (n = 6 per group). The experimental groups; Group I (control), which received corn oil by gavage for 14 days. Group II (chrysin) were received chrysin (100 mg/kg/bw) by gavage for 14 days. Group III (CCl_4) were given CCl_4 0.5 ml/kg/bw in corn oil (1:1) by gavage for 14 days. Group IV (chrysin + CCl_4) were given chrysin and additionally CCl_4 for 14 days. To determine FI, the animals were housed in individual cages. The animals were individually weighed initially and then weekly to monitor their body weight (BW). Additionally, FI and body weight change (BWC) at 7 and 14 days of the experiment were determined.

Sample collection

Twenty-four hours after the last drug administration, blood samples were collected. After the collection of all blood samples were euthanized by cervical dislocation under ether anesthesia. The collected blood samples were centrifuged at 2500 g at 4 °C for 15 min and stored at −20 °C until analysis. The liver and kidney tissue samples were removed for biochemical and histological analyses.

Biochemical Analysis

The malondialdehyde (MDA) level of liver and kidney tissue, superoxide dismutase (SOD) activity, reduced glutathione (GSH) concentration and serum TNF-α levels were determined. Liver and kidney tissues were homogenized in 1/10 ratio with 1.15% KCI. MDA analysis was performed in half of the homogenate. The other half of the homogenate was centrifuged at 2500 g for 45 min at +4 °C, and the separated supernatants were used for SOD and GSH analyses. Protein concentrations in the supernatants were determined by the Lowry method [16], and the results were adapted to the protein. In addition, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzyme activities, as well as urea and creatinine levels were determined using a biochemical autoanalyzer (Siemens Advia 2400, USA).

Measurement of tissue MDA levels

Tissue lipid peroxidation levels were measured by the spectrophotometric method of Ohkawa et al. [17] at 532 nm, according to the concentrations of thiobarbituric acid reactive substances (TBARS), and the level of MDA produced was used as an index of lipid peroxidation. 1,1,3,3-tetraethoxypropane was used as a standard. MDA values were expressed as nmol/ml homogenate.

Measurement of tissue GSH concentrations

GSH levels were determined by the methods of Ellman et al. [18] in tissues. 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB) is a disulfide compound reduced by sulfhydryl compounds.

The color intensity of the yellow complex formed by the sample and DTNB is directly proportional to GSH concentration in the medium. The protein concentrations were determined by the method of Lowry et al. [16], with bovine serum albumin as the standard. GSH levels were measured spectrophotometrically at 412 nm, and expressed as nmol/mg protein.

Measurement of tissue SOD activities

Tissue SOD activities were determined according to the method of Sun et al. [19]. The samples were incubated with xanthine oxidase solution to measure the SOD activity. Absorbance was read at 560 nm to measure superoxide anions. The activity was determined as the inhibition of chromogen reduction. In the presence of SOD, the superoxide anion concentration is reduced, yielding a colorimetric signal. The SOD activity was expressed as percent inhibition.

Serum TNF-α levels

Serum TNF-α levels were determined using an enzyme-linked immunosorbent assay (ELISA) commercial rat kit (Elabscience, USA), according to the manufacturer's instructions. Cytokine quantities in the samples were calculated from the standard curves of recombinant cytokines by using a linear regression method. The results were expressed as pg/ml.

Histopathological and immunohistochemical (IHC) analyses

For histological examinations, the liver and kidney tissues were fixed in formalin solution (10%), dehydrated in graded alcohol series, and embedded in paraffin. The paraffin-embedded tissue samples were cut into 5 μm thick sections, mounted on 1/4 shaven slides and stained with hematoxylin-eosin (H&E) for histological examination, according to a standard procedure [20]. Apoptotic cells in the tissues were evaluated immunohistochemically using the caspase method. For analysis, the sections were mounted on poly-L-lysine-coated slides. Following deparaffinization and rehydration, the samples were transferred to a citrate buffer (pH 6.0), heated in a microwave oven for 20 min, cooled for 20 min at room temperature, and washed with phosphate-buffered saline (PBS). The sections were immersed in 0.3% H_2O_2 for 5 min, washed with PBS, incubated with Ultra V block at room temperature for 5 min, and incubated with a primary rabbit polyclonal active caspase-3 antibody (Thermo Scientific, CPP32, Ab-4, UK) in a humidified chamber at 37 °C for 1 h. The sections were rinsed in PBS before being incubated with a biotinylated goat anti-polyvalent secondary antibody at room temperature for 30 min and then washed with PBS. They were then incubated with streptavidin peroxidase at room temperature and washed with PBS. Staining was completed after the substrate was incubated with a 3,3'-diaminobenzidine tetrahydrochloride (DAB) chromogen (Thermo Scientific, Ultra Vision Detection System Anti-Polyvalent, HRP/DAB, UK) for 5–15 min, and the slides were washed with distilled water for 5 min. The slides were counterstained with Mayer's hematoxylin for 30 s, rinsed in water for 5 min, dehydrated, and mounted with an aqueous mounting medium. H&E tissue sections and active caspase-3 activities in the liver and kidney tissues were examined using a binocular-headed light microscope, which has the features of a photomicroscope (Olympus BX-51, Olympus Optical Co., Ltd., Tokyo, Japan), and images were taken. The active caspase-3 kit was used according to the manufacturer's instructions. Active caspase-3 positive cells in the tissue sections were stained a brown color and evaluated by analyzing at the staining intensity (−; no, +; weak, ++; moderate, +++; strong) using a semi-quantitative scoring system.

Statistical analysis

The SPSS 19.0 (SPSS Inc., Chicago, IL, USA) software was used for all statistical analyses, and all assays were performed at least three times. Data normality was analyzed using the Shapiro Wilk test, and a normal distribution was found. Original data were verified for normality using Q-Q plots and analyzed for the equality of variance using box plots. Differences among the groups were analyzed using one-way analysis of variance (ANOVA), followed by a post hoc Tukey test (equal variances were assumed). All values are presented as the mean ± standard error (SE). Differences were considered significant at $p < 0.05$.

Results

BW, BWC, FI, and Biochemical findings

The effects of chrysin on BW, BWC, and FI in all groups are described in Table 1. The BWC of all groups was not significantly different until 14 days. After this period, it was significantly different ($p < 0.05$). In addition, the effects of chrysin on BW in all groups were not significant. By this study, it was demonstrated that BWC and FI increased when chrysin supplementation was provided to the animals

Table 1. Effects of chrysin on BW, BWC and FI in the experimental groups

	Days	Control	Chrysin	Chrysin + CCl_4	CCl_4	P
BW(g)	IW	183 ± 6.95	181 ± 8.48	187 ± 8.75	188 ± 8.49	NS
	7	197 ± 9.49	196 ± 9.57	192 ± 5.29	192 ± 5.85	NS
	14	214 ± 13.7	212 ± 9.15	198 ± 5.86	195 ± 7.59	NS
BWC(g)	1–7	2.40 ± 0.59	2.38 ± 0.62	0.83 ± 0.17	0.68 ± 0.10	NS
	8–14	2.48 ± 0.61	2.36 ± 0.41	0.85 ± 0.21	0.42 ± 0.30	NS
	1–14	2.44 ± 0.57[a]	2.37 ± 0.37[a]	0.84 ± 0.21[ab]	0.54 ± 0.20[b]	*
FI(g)	1–7	23.6 ± 0.60[a]	23.5 ± 0.82[a]	12.1 ± 0.78[b]	11.3 ± 0.42[b]	***
	8–14	22.2 ± 1.11[a]	22.1 ± 0.68[a]	11.9 ± 0.83[b]	9.78 ± 0.52[b]	***
	1–14	22.9 ± 0.76[a]	22.8 ± 0.44[a]	12.0 ± 0.70[b]	10.5 ± 0.95[b]	***

a, b: Mean values with different superscripts within a row differ significantly, *: $p < 0.05$; ***: $p < 0.001$.
NS: Non significant, *IW*: Initial weight, *BW*: Body weight, *BWC*: Body weight change, *FI*: Feed intake (per day)

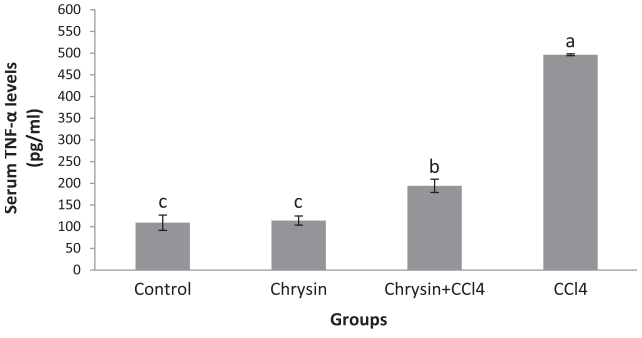

Figure 1. Effects of chrysin on serum TNF-α levels in the experimental groups. *a, b, c*: Differences between groups in the same row including different letters are significant. (n = 6 per group, *bars represents standard error*). *TNF-α*; tumor necrosis factor-α, *CCl_4*; carbon tetrachloride.

treated with CCl_4. The FI decreased significantly in CCl_4 group compared with the other groups ($p < 0.001$). The FI of control and chrysin group animals were found to be similiar. The supplementation of chrysin significantly increased the FI in the CCl_4-treated group.

The levels of serum TNF-α are given in Figure 1. Serum TNF-α levels in the CCl_4 group were significantly ($p < 0.05$) higher than the control group. In addition, the treatment of chrysin significantly decreased serum TNF-α levels ($p < 0.05$). The MDA, GSH, and SOD activitiy levels in the liver and kidney tissues are presented in Table 2. The tissue MDA levels were significantly higher ($p < 0.05$) in the CCl_4 group than in the control group. Treatment with chrysin significantly decreased MDA levels ($p < 0.05$). In addition, the liver and kidney tissues GSH levels in the CCl_4 group were significantly ($p < 0.05$) lower than the control group. Treatment with chrysin significantly increased the levels of GSH in tissues ($p < 0.05$). Furthermore, SOD activity in the CCl_4 group was significantly ($p < 0.05$) lower than that in the control group. Treatment with chrysin significantly increased SOD activity ($p < 0.05$). Serum AST, ALT, urea, and creatinine levels are shown in Table 3. Serum AST, ALT, urea, and creatinine levels were significantly ($p < 0.05$) higher in the CCl_4 group than in the control group. In addition, treatment with chrysin decreased AST ($p < 0.05$), ALT ($p < 0.05$), urea, and creatine ($p < 0.05$) levels.

Histopathological and immunohistochemical findings

Histopathological and immunohistochemical results in the kidney and liver tissues are presented in the Figures 2–5. The control and chrysin groups demonstrated normal liver histology (Figure 2A, 2B). In these groups, the liver tissues had a normal structure with no abnormalities. In contrast, the liver tissues in the CCl_4 group (Figure 2C, 2D) demonstrated congestion and dilatation of central veins with severe hepatocellular damage represented by diffuse microvesicular and macrovesicular hepatic steatosis and ballooning degeneration of hepatocytes. Furthermore, an enlargement of hepatocytes and extensive centrilobular necrosis were observed. It was also determined that the focal mononuclear inflammatory cellular aggregation in hepatic parenchyma, vasocongestion in sinusoids, and hemorrhage were caused by CCl_4 exposure. The treatment of chrysin alleviated the CCl_4-induced these histopathological changes, and mild hepatic lesions were observed in the liver architecture (Figure 2E, 2F).

In addition, the control and chrysin groups demonstrated no abnormalities in the kidney histology as represented respectively (Figure 3A, 3B). The kidney tissues in the CCl_4 group exhibited dilatation and an increase in Bowman's space with glomerular shrinkage, destruction, and atrophy in the cortex region (Figure 3C, 3D). A higher number of capillaries were seen in some glomeruli. Tubular degeneration and dilatation, as well as intraluminal cellular debris, were also present in some tubules. Furthermore, vascular congestion, mononuclear inflammatory cell infiltration, and distortion of renal corpuscles were observed. The treatment of chrysin decreased the CCl_4-

Table 2. The effects of chrysin on MDA and GSH levels, SOD activities in the tissues of the experimental groups

Tissues	Control	Chrysin	Chrysin + CCl_4	CCl_4
Liver				
MDA (nmol/ml of homogenate)	3.03 ± 0.13^{c}	2.83 ± 0.17^{c}	4.54 ± 0.18^{b}	9.68 ± 0.17^{a}
GSH (nmol/mg protein)	79.8 ± 2.30^{a}	76.2 ± 2.03^{a}	64.1 ± 1.85^{b}	56.8 ± 1.17^{c}
SOD (% inhibition)	32.3 ± 1.37^{a}	30.9 ± 1.50^{a}	29.2 ± 1.06^{b,c}	25.1 ± 1.38^{c}
Kidney				
MDA (nmol/ml of homogenate)	2.20 ± 0.02^{c}	2.32 ± 0.02^{c}	2.97 ± 0.17^{b}	4.49 ± 0.17^{a}
GSH (nmol/mg protein)	44.0 ± 1.87^{a}	42.0 ± 1.65^{a}	31.3 ± 1.41^{c}	31.6 ± 1.39^{b}
SOD (% inhibition)	56.1 ± 1.16^{a}	54.7 ± 1.08^{a}	49.1 ± 1.49^{b}	44.6 ± 1.38^{c}

a, b, c: Differences between groups in the same row including different letters are significant.

Table 3. The effects of chrysin on serum AST, ALT, urea, and creatinine levels in the experimental groups

	Control	Chrysin	Chrysin + CCl_4	CCl_4
AST (U/L)	240 ± 12.9^{c}	217 ± 15.1^{c}	312 ± 15.8^{b}	511 ± 14.5^{a}
ALT (U/L)	84.6 ± 4.24^{b}	89.8 ± 2.39^{b}	102 ± 5.95^{b}	182 ± 3.94^{a}
Urea (mg/dL)	62.0 ± 1.44^{b}	64.2 ± 1.65^{b}	69.1 ± 2.71^{a,b}	81.3 ± 2.03^{a}
Creatinine (mg/dL)	0.21 ± 0.009^{c}	0.20 ± 0.001^{c}	0.29 ± 0.001^{b}	0.37 ± 0.002^{a}

a, b, c: Differences between groups in the same row including different letters are significant.

induced these histopathological changes, and mild renal lesions were seen in the kidney architecture (Figure 3E, 3F). The liver and kidney tissues of control and chrysin groups revealed no active caspase-3 immunoreactivity (Figure 4A, 4B and 5A, 5B). Apoptotic cells showing a strong positive reaction were observed in the hepatocytes of proximal and distal tubules in the CCl_4 group (Figure 4C, 4D and 5C, 5D). However, the treatment of chrysin reduced the CCl_4-induced apoptotic cells, and the staining intensities of these apoptotic cells were moderate in the liver and kidney tissues (Figure 4E, 4F and 5E, 5F).

Discussion

Flavonoids are phenolic compounds that are the most common chemical class of phytochemicals. They are known to possess a wide range of health promoting effects. As a natural flavonoid, chrysin is commonly found in propolis and honey. Previous studies demonstrated that chrysin exhibits protective effects in different animal tissues, such as the brain, heart, liver, kidney, and lungs [21]. Chrysin mediates its anti-inflammatory activity by blocking histamine release and inducing proinflammatory cytokine expression [22, 23]. It also shows anticancer activity by promoting cell death induced by TNF-related apoptosis inducing ligand (TRAIL) and increasing TRAIL-induced degradation of caspases-3 and caspases-8, 10, 25 inhibition of TNF-α and IL-1b [22, 24, 25]. In addition, several animal studies demonstrated that chrysin acts as an antidote in different intoxications induced by toxic agents [21]. CCl_4 is a known potent, lipid soluble, hepatotoxic agent. CCl_4 is biotransformed to the trichloromethyl radical by the cytochrome P450 system in liver microsomes [26]. As a consequence, it causes lipid peroxidation of membranes that leads to liver injury. Because the basic structures of human and rat livers are similar, the administration of CCl_4 is an accepted experimental model for inducing hepatic damage [26, 27], and it has been commonly used in experimental models to elucidate the cellular mechanisms behind oxidative damage [28]. As a well-known hepatotoxic agent, CCl_4 exhibits its toxic effects via the generation of free radicals, such as hydroxyl ethyl radicals, superoxide radicals, hydroxyl radicals, peroxyl radicals, and hydrogen peroxide [29]. Because of the lipid solubility of CCl_4, it can cross cell membranes. CCl_4 is distributed and deposited in organs such as the liver, kidney, and brain, after treatment. The toxicity of CCl_4 likely depends on the formation of the trichloromethyl radical, which interacts with oxygen to form CCl_3O_2 [4, 26]. Chrysin prevents CCl_4-induced renal toxicity by increasing the levels of GSH, CAT, GPx, and SOD and decreasing lipid peroxidation and expression of the inducible nitric oxide synthase (iNOS) gene [22].

In this study, FI ($p < 0.001$) and BWC ($p < 0.05$) in the CCl_4 group decreased significantly in comparison with the other groups for 14 days. Consistent with this study, Junnila et al. [30] reported that exposure to CCl_4 (1 ml/kg/day subcutaneously for 4 consecutive days) significantly reduced the average FI and growth rates of rats. Similarly, Behboodi et al. [31] reported that CCl_4 significantly reduced FI and BW gain in Japanese quails subjected CCl_4-induced oxidative stress. Reduced BW gain could be linked to reduced nutrient digestion and

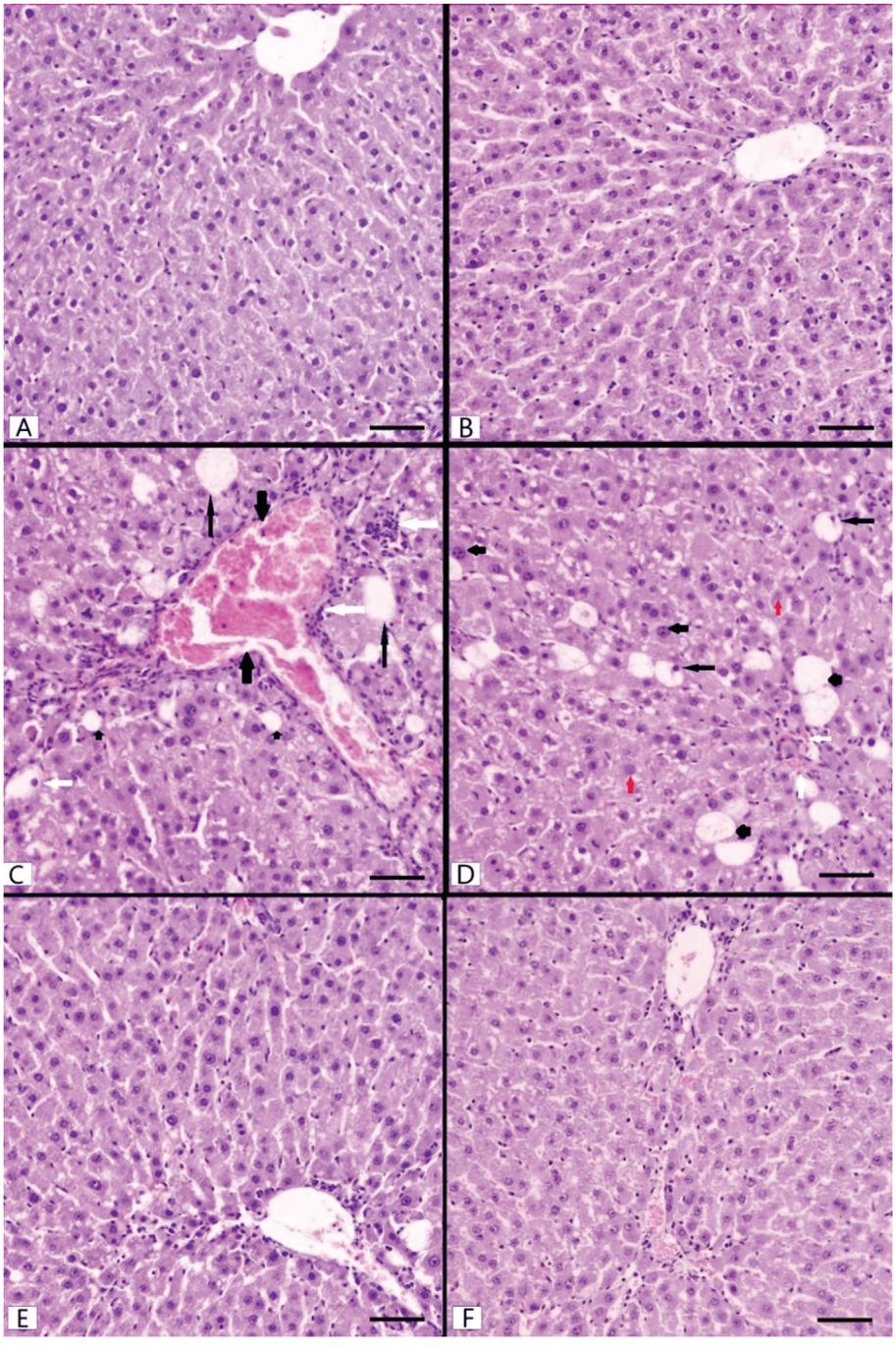

Figure 2. Histopathological changes in the liver tissues of the experimental rats (H&E staining). (A) *Control*; normal morphology, (B) *Chrysin*; normal morphology, (C) *CCl_4*; congestion and dilatation of the central vein (black thick arrows), microvesicular steatosis (arrowheads), macrovesicular steatosis (black thin arrows), ballooning cell (white small arrow), focal inflammatory cell infiltration (white big arrows), (D) *CCl_4*; enlargement of hepatocytes (black small arrows), necrotic cells (red arrows), vasocongestion of sinusoids (white arrows), ballooning cells (black big arrows), macrovesicular steatosis (arrowheads), (E) *Chrysin + CCl_4*; mild hepatic lesions, (F) *Chrysin + CCl_4*; mild hepatic lesions. *Scale bars: 50 μm.*

absorption because of low bile acid secretion [32]. In addition, Khodadust et al. [33] reported the worsened effect of CCl_4 on the performance of broiler chicken. Our study demonstrated that FI and BWC significantly increased when chrysin supplementation was provided to CCl_4-treated rats. The beneficial effects of chrysin on performance could be linked to its flavonoid content [34]. In accordance with this study, Anand et al. [26] demonstrated that chrysin (200 mg/kg bw) was able to prevent oxidative damage induced by intraperitoneal administration of CCl_4 (2 ml/kg) in the liver, brain, kidney and hemolysate of male Wistar rats. In another study, Tatli Seven et al.

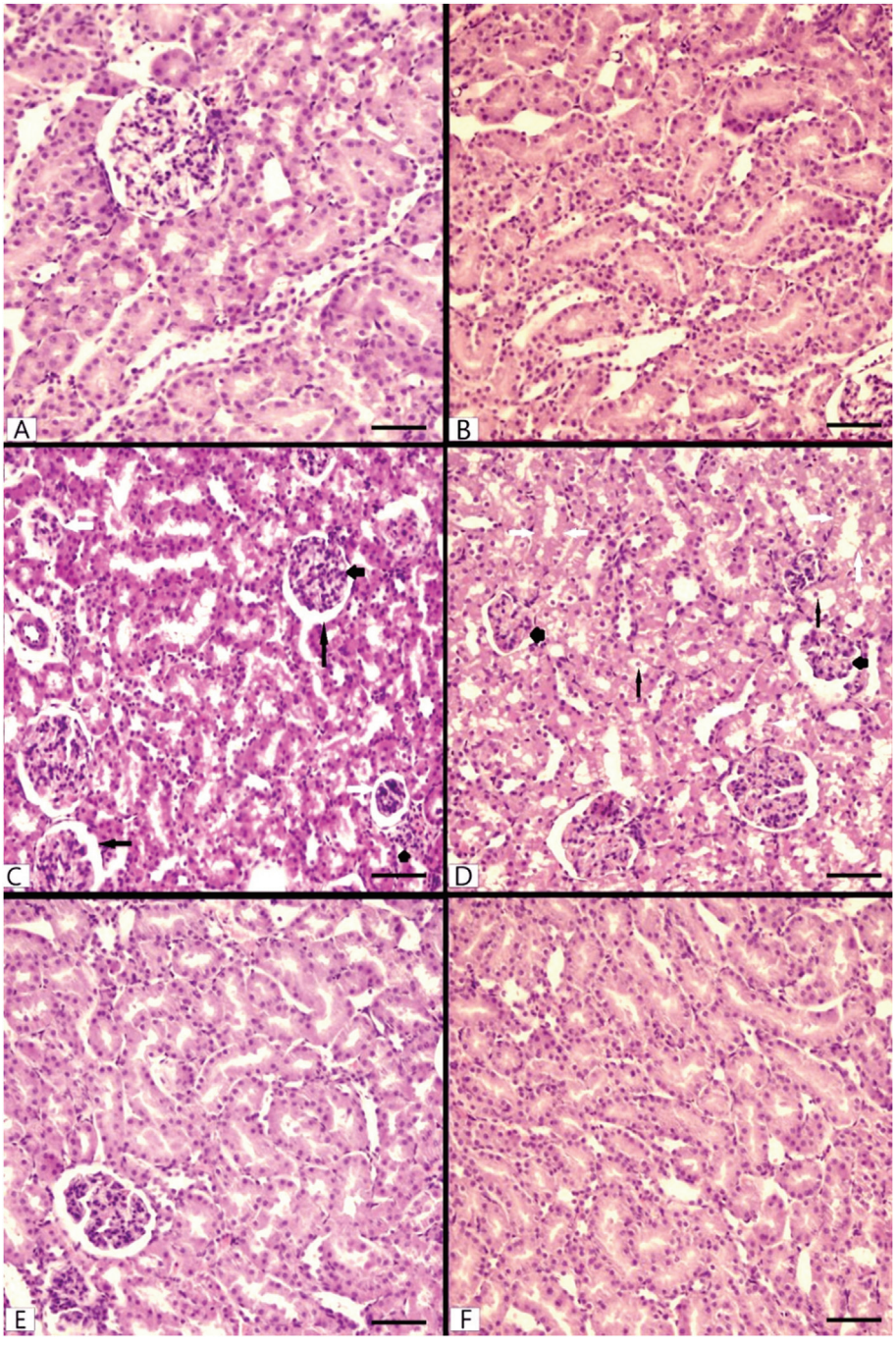

Figure 3. Histopathological changes in the kidney tissues of the experimental rats (H&E stain). (A) *Control*; normal morphology, (B) *Chrysin*; normal morphology, (C) *CCl_4*; dilatation and increase of Bowman's space (black big arrows) with glomerular shrinkage (black small arrow), glomerular destruction and atrophy (white arrows), vascular congestion and inflammatory cell infiltration (arrowhead), (D) *CCl_4*; tubular degeneration and dilatation (white arrows), intraluminal cellular debris in some tubules (black arrows), distortion of renal corpuscles (arrowheads), (E) *Chrysin + CCl_4*; mild renal lesions, (F) *Chrysin + CCl_4*; mild renal lesions. *Scale bars: 50 μm.*

[35] demonstrated that chrysin (50 ppm) appeared to ameliorate the adverse effects caused by Cu toxicity in rats. According to these results, they suggested that chrysin treatment alleviated degeneration, necrosis, and apoptosis in the liver and kidney tissues of the Cu-treated rats. One of the reasons contributing oxidative stress caused by ROS is CCl_4 induces chemical liver injury [36, 37]. Lipid peroxidation is a considerable indicator of oxidative stress [37]. A crucial end product of lipid peroxidation in hepatic oxidative stress is MDA. The level of MDA could be used

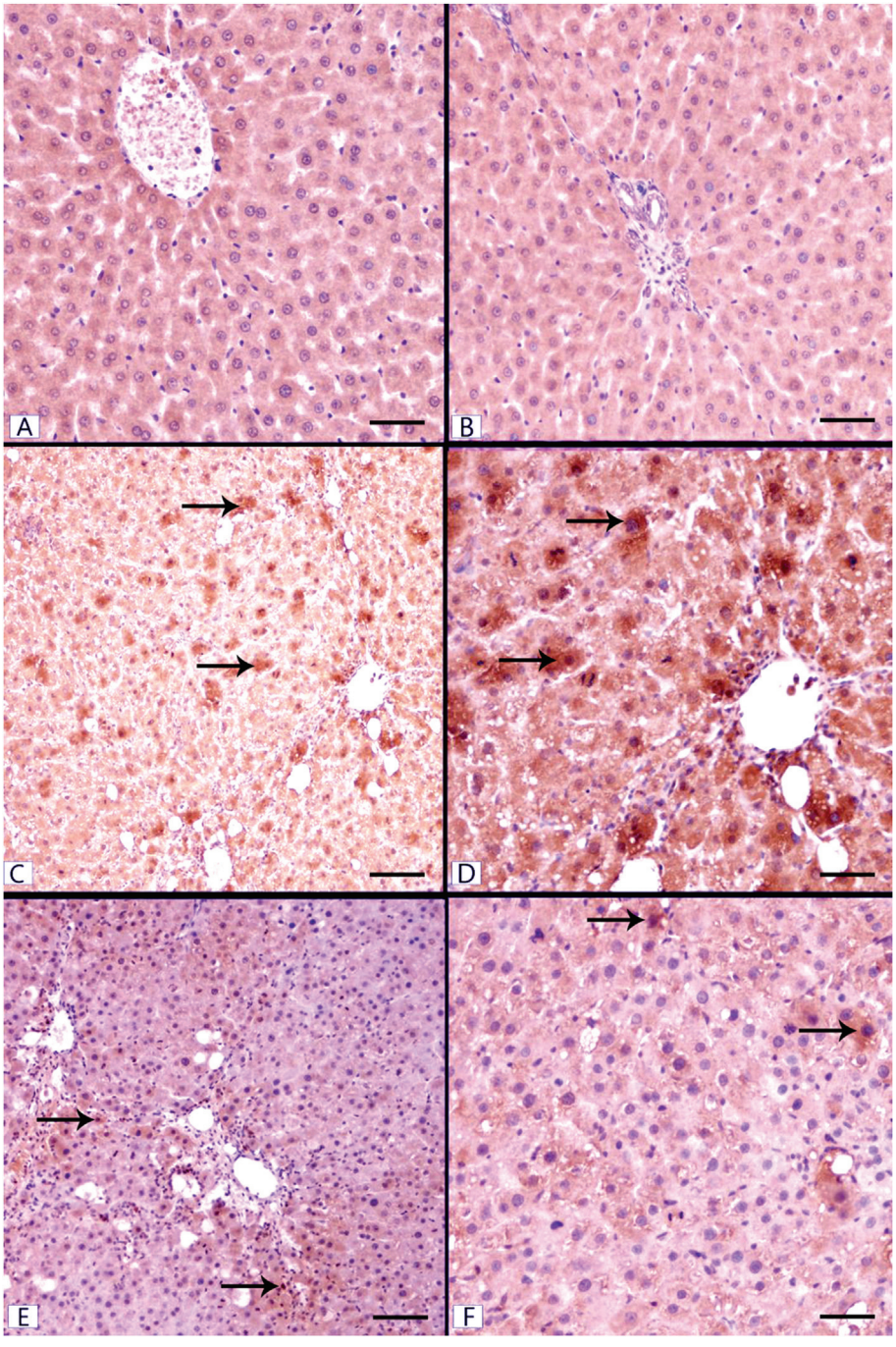

Figure 4. Immunohistochemical findings in the liver tissues of rats (IHC, Mayer's hematoxylin counterstain). (A) *Control*; no immunoreactivity for active caspase-3 antibody, (B) *Chrysin*; no immunoreactivity for active caspase-3 antibody, (C) *CCl_4*; numerous apoptotic cells showing strong immunoreactivity for active caspase-3 (arrows), (D) *CCl_4*; numerous apoptotic cells showing strong immunoreactivity for active caspase-3 (arrows), (E) *Chrysin + CCl_4*; decreased numbers of active caspase-3 immunopositive cells with moderate staining intensities (arrows), (F) *Chrysin + CCl_4*; decreased numbers of active caspase-3 immunopositive cells with moderate the staining intensities (arrows). *Scale bars: 20 μm (A, B, D and F) and 50 μm (C and E).*

as an indicator of CCl_4-induced liver injury [38]. In this study, MDA levels in kidney and liver tissues were found to be high ($p < 0.05$). Increased MDA levels as a result of CCl_4 induced liver injury were also demonstrated by Jadhav et al. [7], Anand et al. [26], Zhang et al. [37], and Lu Et al. [39]. The result of this study showed that chrysin supplementation significantly decreases MDA levels ($p < 0.05$). This effect could be linked to the ability of chrysin to increase the levels of antioxidants, along with its anti-lipid peroxidative activity. Additionally, chrysin

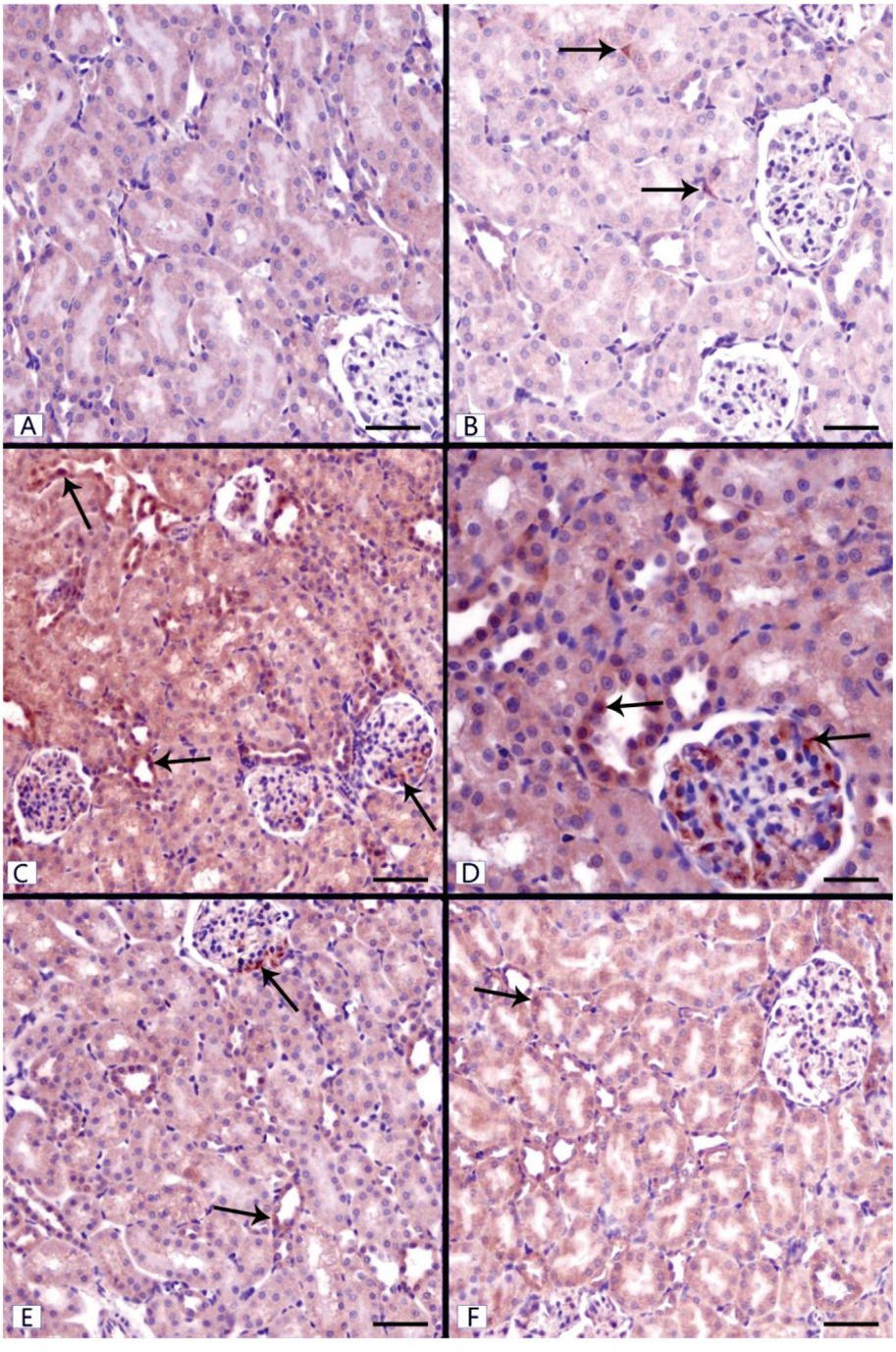

Figure 5. Immunohistochemical findings in the kidney tissues of rats (IHC, Mayer's hematoxylin counterstain). (A) *Control*; no immunoreactivity for active caspase-3 antibody, (B) *Chrysin*; no immunoreactivity for active caspase-3 antibody (arrows), (C) CCl_4; numerous apoptotic tubular epithelial cells showing strong immunoreactivity for active caspase-3 (arrows), (D) CCl_4; numerous apoptotic tubular epithelial cells showing strong immunoreactivity for active caspase-3 (arrows), (E) *Chrysin* + CCl_4; decreased numbers of active caspase-3 immunopositive tubular cells with moderate staining intensities (arrows), (F) *Chrysin* + CCl_4; decreased numbers of active caspase-3 immunopositive tubular cells with moderate the staining intensities (arrows). *Scale bars: 20 μm (A, B and D) and 50 μm (C, E and F).*

could be potentially useful in counteracting the free radical-mediated injury involved in the development of tissue damage caused by CCl_4 [26].

As an important intracellular antioxidant, GSH plays several vital roles, such as antioxidation, maintenance of the redox state, xenobiotic detoxification, and cellular protection from damage by free radicals, peroxides, and toxins [37, 40, 41]. SOD is an important antioxidant enzyme that reacts rapidly and is removed by CAT as it converts O_2 into H_2O_2 [38, 42]. Reduction of CAT, SOD, and GPx

activities is reported to be related to the accumulation of highly reactive free radicals, leading to damaging effects, such as the loss of integrity and function of cell membranes [43]. In this study significantly decreased levels of GSH and SOD were obseved in the CCl_4 group ($p < 0.05$). However, chrysin supplementation increased GSH and SOD levels in the CCl_4 + chrysin group. These results are consistent with those of the study by Anand et al. [26]. This protective effect of chrysin could be linked to the decreased production of CCl_4-induced free radicals [26].

Increased activities of serum AST and ALT were observed in the CCl_4 group compared with other groups in our study. Similar results have been reported by other studies [3, 7, 26, 44–47]. AST and ALT activities are used as biochemical markers of liver damage [37]. Molehina et al. [46] reported that the elevated level of cytosolic liver markers, such as AST, and ALT, may be associated with them leaking out from swollen and necrotic hepatocytes into the blood circulation. Chrysin supplementation significantly decreased serum AST and ALT activity in the CCl_4 group. This result is consistent with the findings of Anand et al. [26] and Hermenean et al. [45]. The decreased levels of AST and ALT could be linked to the ability of chrysin to protect the structure of membranes [45].

In this study, the urea and creatinine levels were significantly increased ($p < 0.05$) in the CCl_4 groups compared with the other groups. CCl_4 is a nephrotoxic substance. CCl_4 administration leads to nephrotoxicity, as indicated by an elevation in serum levels of urea, creatinine, and uric acid [47]. Similar results were obtained by Hwang et al. [44], Mbarki et al. [48], and Mazani et al. [49]. It was reported that CCl_4 supplementation caused renal toxicity, evidenced by increased serum levels of creatinine and urea. Increased levels of creatinine and urea may be signs of severe damage to the structural integrity of nephrons [47, 48]. The protective effect of chrysin against chemical-induced renal toxicity was reported in previous studies [26, 50]. Consistent with our study, Anand et al. [26] reported that chrysin prevented CCl_4-induced oxidative damage in rat kidney.

Oxidative stress and inflammatory cytokines are closely related to each other and play an essential role in chemical and drug induced acute kidney damage [51]. TNF-α is a pro-inflammatory cytokine produced by Kupffer cells. It has been found to be elevated in acute liver diseases and following exposure to hepatotoxic chemicals, such as CCl_4 [45]. CCl_4 leads to the production of toxic trichloromethyl and trichloromethyl peroxyl radicals, which may be responsible for the generation of cytokines, such as IL-1β, IL-2, and TNF-α [52]. According to this study, CCl_4-induced histopatological changes confirmed the antioxidant status and TNF-α level. TNF-α levels were significantly higher ($p < 0.05$) (Table 2) in the CCl_4 group than those in the other groups. In accordance with this study, Tatli Seven et al. [35] demonstrated that serum TNF-α levels were significantly higher ($p < 0.001$) in the group that was administrated copper sulfate (Cu; 500 ppm) than those in the other groups. Serum TNF-α levels were significantly low in the Cu + flunixin meglumine and Cu + chrysin groups compared with the Cu group. They suggested that decreased TNF-α levels could be because of the flavonoid content of chrysin. In a previous study, Hermenean et al. [45] reported that the hepatoprotective activity of chrysin was identified to be mediated through TNF-α. Specifically, chrysin reduced soluble TNF-α generation by inhibiting the TNF-α converting enzyme.

Cell death may occur in organisms which programmed sequence of events leads to the elimination of cell without releasing detrimental substance in the surrounding area via apoptosis. Apoptosis plays a role in many diseases by its stimulation and inhibition. Cell death arises from the activation of the initiator caspase (caspase-9) that consequently activates the executioner caspase (caspase-3), which eventually kills the cell by protein degradation [52]. Caspase 3 activity was significantly increased in the CCl_4 treatment group in this study. This is consistent with the study by Safhi [52], which reported an increase in caspase-3 activity in CCl_4-treated rats. Similarly, Osman et al. [53] reported that the liver of CCl_4-supplemented rabbits exhibited significant increases in iNOS and caspase-3 expressions. CCl_4 treatment caused serious hepatic changes due to the enhancement of oxidative stress and the induction of apoptosis. Apoptosis in hepatocytes arises from toxin-induced extreme oxidative stress, exhaustion of antioxidant enzymes, and induction of membrane lipid peroxidation.

In this study, we observed that CCl_4-supplemented rats exhibited congestion and dilatation of central veins with severe hepatocellular damage represented by diffuse microvesicular and macrovesicular hepatic steatosis and ballooning degeneration of hepatocytes. The results of histological examinations of this study are similar to those of previous studies [48, 54, 55]. Rats treated with CCl_4 demonstrated significant congestion of the sinusoids, enlargement of hepatocytes, and vacuolization (foamy or ballooning degeneration) in the cytoplasm with extensive fatty change [48]. Similarly, serious effects were reported by Lee et al. [54] and Fahmy et al. [55], including the concavity of the surface of the liver, necrosis, ballooning degeneration, fibrosis, and lymphocyte infiltration in the central vein. Likewise, Osman et al. [53] reported that the CCl_4-induced hepatotoxicity in rabbits was characterized by serious hepatocellular damage and mononuclear cellular infiltration of the hepatic necrotic areas. Histological examination vascular congestion, mononuclear inflammatory cell infiltration, and distortion of renal corpuscles were seen in rats

caused by CCl_4. Hwang et al. [44] demonstrated that CCl_4 treatment caused an increase in the diameter of the glomerulus, as well as a higher number of capillaries in the glomerulus. Mbarki et al. [48] observed that CCl_4 treatment caused different effects on the kidney, such as glomerular necrosis, tubular epithelial cell changes, and histological changes of the proximal and distal tubules. These histological changes likely originated from the products of lipid peroxidation and the destruction of membranes [48].

The results of this study show that CCl_4 is a potential hepatotoxic and nephrotoxic chemical. CCl_4 caused oxidative stress by exhausting the activities of antioxidant enzymes, producing inflammatory cytokines, and stimulating apoptosis. Chrysin supplementation appeared to ameliorate the CCl_4-induced adverse effects on FI by increasing antioxidant activities and scavenging the free radicals. Therefore, chrysin may be a promising agent to treat some diseases that causes liver or kidney injury.

Although our study indicated the protective effects of chrysin on lipid peroxidation, TNF-α levels, and liver and kidney function in rats with CCl_4-induced liver and kidney injury, it has some limitations. Firstly, the study was performed on a relatively small groups of animals. Therefore, further studies are necessary to confirm our results. Moreover, because ether anesthesia administrated to the rats in ours experiment had the ability to cause the changes in serum levels. Thus, we used a saline group to reduce the risk of false positive results.

References

1. Tur L. Investigation of liver protective effects of matricaria chamomilla l. on liver damage induced by carbon tetrachloride. Afyon Kocatepe Univ Inst Health Sci. 2008.
2. Abraham P, Wilfred G, Cathrine SP. Oxidative damage to the lipids and proteins pf the lungs, testis and kidney of rats during carbon tetrachloride intoxication. Clin Chim Acta. 1999;289:177–179.
3. Tirkey N, Pilkhwal S, Kuhad A, Chopra K. Hesperidin, a citrus bioflavonoid, decreases the oxidative stress produced by carbontetrachloride in rat liver and kidney. BMC Pharmacol. 2005;5(2):1–8.
4. Parola M, Robino G. Oxidative stress-related molecules and liver fibrosis. J Hepat. 2001;35(2):297–306.
5. Wang H, Wei W, Wang NP, Gui SY, Wu L, Sun WY, et al. Melatonin ameliorates carbon tetracholoride-induced hepatic fibrogenesis in rats via inhibition of oxidative stress. Life Sci. 2005;77:1902–1915.
6. Jadhav VB, Thakare VN, Suralkar AA, Deshpande AD, Naik SR. Hepatoprotective activity of luffa acutangula against CCl4 and rifampicin induced liver toxicity in rats: a biochemical and histopathological evaluation. Indian J Exp Biol. 2010;48:822–829.
7. Manibusan MK, Odin M, Eastmond DA. Postulated carbon tetrachloride mode of action: a review. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2007;25:185–209.
8. Dundar Y. Phytochemicals and healthy life. The MedJ Kocatepe. 2001;2:131–138.
9. Sultana S, Verma K, Khan R. Nephroprotective efficacy of chrysin against cisplatin-induced toxicity via attenuation of oxidative stres cisplatin. J Pharm Pharmacol. 2012;64:872–881.
10. Pulido MD, Parrish AR. Metal-induced apoptosis: mechanisms. Mutat Res. 2003;533:227–241.
11. Wang BJ, Lien YH, Yu ZR. Supercritical fluid extractive fractionation study of the antioxidant activities of propolis. Food Chem. 2004;86:237–243.
12. Pushpavalli G, Kalaiarasi P, Veeramani C, Pugalendi KV. Effect of chrysin on hepatoprotective and antioxidant status in D-galactosamine-induced hepatitis in rats. Eur J Pharmacol. 2010;631:36–41.
13. Cho H, Yun CW, Park WK, Kong JY, Kim KS, Park Y, Lee S, Kim BK. Modulation of the activity of pro-inflammatory enzymes, COX-2 and iNOS, by chrysin derivatives. Pharmacol Res. 2004;49(1):37–43.
14. Sathiavelu J, Senapathy GJ, Devaraj R, Namasivayam N. Hepatoprotective effect of chrysin on prooxidant-antioxidant status during ethanol-induced toxicity in female albino rats. J Pharm Pharmacol. 2009;61(6):809–817.
15. Ciftci O, Ozdemir I, Aydin M, Beytur A. Beneficial effects of chrysin on the reproductive system of adult male rats. Andrologia. 2012;44(3):181–186.
16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with pholin phenol reagent. J Biol Chem. 1951;193:265–275.
17. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95:351–358.
18. Ellman G. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82:70–77.
19. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem. 1988;34:497–500.
20. Ross MH, Reith EJ, Romrell LJ. Histology: a text and atlas. (2nd ed). Baltimore: Williams and Wilkins; 1989.
21. Samarghandian S, Farkhondeh T, Azimi-Nezhad M. Protective effects of chrysin against drugs and toxic agents. Dose-Response. 2017;15(2):1–10.
22. Samarghandian S, Afshari JT, Davoodi S. Chrysin reduces proliferation and induces apoptosis in the human prostate cancer cell line pc-3. Clinics (Sao Paulo). 2011;66(6):1073–1079.
23. Bae Y, Lee S, Kim SH. Chrysin suppresses mast cell-mediated allergic inflammation: involvement of calcium, caspase-1 and nuclear factor-kB. Toxicol Appl Pharmacol. 2011;254(1):56–64.
24. Li X, Wang JN, Huang JM, Xiong XK, Chen MF, Ong CN, Shen HM, Yang XF. Chrysin promotes tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) induced apoptosis in human cancer cell lines. Toxicol In Vitro. 2011;25(3):630–635.
25. Bai J, Luo Y, Zhanchun S, Fan W, Wang Z, Luan T, Jiang J, Zang B. Effects and the mechanisms of chrysin on sepsis-associated acute lung injury of rats chrysin inhibits acute lung injury. Life Sci J. 2013;10(3):1052–1058.
26. Anand KV, Anandhi R, Pakkiyaraj M, Geraldine P. Protective effect of chrysin on carbon tetrachloride (CCl4)-induced tissue injury in male wistar rats. Toxicol Ind Health. 2011;27 (10):923–933.
27. Kogure K, Ishizaki M, Nemoto M, Kuwano H, Makuuchi M. A comparative study of the anatomy of rat and human livers. J Hepatobiliary Pancreat Surg. 1999;6:171–175.
28. Basu S. Carbon tetrachloride induced lipid peroxidation: eicosanoid formation and their regulation by antioxidant and nutrients. Toxicol. 2003;189:113–127.
29. Michiels C, Raes M, Toussaint O, Remacle J. Importance of se-glutathione peroxidase, catalase and Cu/Zn-SOD for cell survival against oxidative stress. Free Radic Biol Med. 1994;17:235–248.

30. Junnila M, Rahko T, LA SukuraA Lindberg. Reduction of carbon tetrachloride-induced hepatotoxic effects by oral administration of betaine in male han-wistar rats: a morphometric histological study. Vet Pathol. 2000;37:231–238.
31. Behboodi HR, Samadi F, Shams Shargh M, Ganji F, Samadi S. Effects of silymarin on growth performance, internal organs and some blood parameters in Japanese quail subjected to oxidative stress induced by carbon tetrachloride. Poult Sci J. 2017;5(1):31–40.
32. Panovska TK, Kulevanova S, Gjorgoski I, Bogdanova M, Petrushevska G. Hepatoprotective effect of the ethyl acetate extract of teucrium polium l. against carbontetrachloride-induced hepatic injury in rats. Acta Pharm. 2007;57:241–248.
33. Khodadust MR, Samadi F, Ganji F, Jafari AY, Asadi GH. Effects of peppermint (Mentha piperita L.) alcoholic extract on carbon tetrachloride-induced hepatotoxicity in broiler chickens under heat stress condition. Poult Sci J. 2015;3(1):1–16.
34. Kasala ER, Bodduluru LN, Barua CC, Madhana RM, Dahiya V, Budhani MK, Mallugari RR, Maramreddy SR, Gogoi R. Chemopreventive effect of chrysin, a dietary flavone against benzo(a)pyrene induced lung carcinogenesis in Swiss albino mice. Pharmacol Rep. 2016;68:310–318.
35. Tatli Seven P, Gul Baykalir B, Seven I, Parlak Ak T, Basak N, Gulluoglu H. The protective effects of chrysin and flunixin meglumine against excess copper in male rats. Turk J Vet Anim Sci. 2018;42:376–387.
36. Lee IC, Kim SH, Baek HS, Moon C, Kang SS, Kim SH, et al. The involvement of Nrf2 in the protective effects of diallyl disulfide on carbon-tetrachloride-induced hepatic oxidative damage and inflammatory response in rats. Food Chem Toxicol. 2014;63:174–185.
37. Zhang Q, Hu X, Hui F, Song Q, Cui C, Wang C, et al. Ethanol extract and its dichloromethane fraction of alpinia oxyphylla miquel exhibited hepatoprotective effects against CCl-4 induced oxidative damage in vitro and in vivo with the involvement of Nrf2. Biomed Pharmacother. 2017;91:812–822.
38. Cheng N, Ren N, Gao H, Lei X, Zheng J, Cao W. Antioxidant and hepatoprotective effects of Schisandra chinensispollen extracton CCl1 induced acute liver damage in mice. Food Chem Toxicol. 2013;55:234–240.
39. Lu Y, Hu D, Ma S, Zhao X, Wang S, Wei G, et al. Protective effect of wedelolactone against CCl4-induced acute liver injury in mice. Int Immunopharmacol. 2016;34:44–52.
40. He J, Huang B, Ban X, Tian J, Zhu L, Wang Y. In vitro and in vivo antioxidant activity of the ethanolic extract from meconopsis quintuplinervia. J Ethnopharmacol. 2012;141:104–110.
41. Shen B, Chen H, Shen C, Xu P, Li J, Shen G, et al. Hepatoprotective effects of lignans extract from herpetospermum caudigerum against CCl4-induced acute liver injury in mice. J Ethnopharmacol. 2015;164:46–52.
42. Ozcelik D, Uzun H. Copper intoxication; antioxidant defenses and oxidative damage in rat brain. Biol Trace Elem Res. 2009;127:45–52.
43. Reedy AC, Lokesh BR. Studies on spice principle as an antioxidant in the inhibition of lipid peroxidation of rat liver microsomes. Mol Cell Biochem. 1992;111:117–124.
44. Hwang IS, Kim JE, Lee YJ, Kwak MH, Choi YH, Kang BC, et al. Protective effects of gomisin A isolated from schisandra chinensis against CCl4-induced hepatic and renal injury. Int J Mol Med. 2013;31:888–898.
45. Hermenean A, Mariasiu T, Navarro-Gonzalez I, Vegara-Meseguer J, Miutescu E, Chakraborty S, et al. Hepatoprotective activity of chrysin is mediated through TNF-α in chemically-induced acute liver damage: An in vivo study and molecular modeling. Exp TherMed. 2017;13:1671–1680.
46. Molehina OR, Oleyedea OI, Idowub KA, Adeyanjuc AA, Olowoyeyea AO, Tubia OI, et al. White butterfly (Clerodendrum volubile) leaf extract protects against carbon tetrachloride-induced hepatotoxicity in rats. Biomed Pharmacother. 2017;96:924–929.
47. Mbarkia S, Alimib Bouzennaa H, Elfekia A, Hfaiedha N Phytochemical study and protective effect of Trigonella foenum graecum (Fenugreek seeds) against carbon tetrachloride-induced toxicity in liver and kidney of male rat. Biomed Pharmacother. 2017;88:19–26.
48. Elsawy H, Badr GM, Sedky A, Abdallah BM, Alzahrani AM, Abdel-Moneim AM Rutin ameliorates carbon tetrachloride (CCl4)-induced hepatorenal toxicity and hypogonadism in male rats. Peer J. 2019;1–20.
49. Mazani M, Mahmoodzadeh Y, Chinifroush Asl MM, Banaei S, Rezagholizadeh L, Mohammadnia A Renoprotective effects of the methanolic extract of Tanacetum parthenium against carbon tetrachloride-induced renal injury in rats. Avicenna J Phytomed. 2018;8(4):370–379.
50. Badreldin HA, Aham SA, Al Za'abi M, Waly MI, Yasin J, Nemmar A, et al. Ameliorative effect of chrysin on adenine-induced chronic kidney disease in rats. PLoS One. 2015;1–14.
51. Xu M, Shi H, Liu D Chrysin protects against renal ischemia reperfusion induced tubular cell apoptosis and inflammation in mice. Exp Ther Med. 2019;17:2256–2262.
52. Safhi MM, Nephroprotective effect of zingerone against CCl4-induced renal toxicity in Swiss albino mice: molecular mechanism. Oxid Med Cell Longev. 2018;1–8.
53. Osman IH, Tantawy AA, Ibrahim HM, El MoustafaAbd GA. Antioxidant properties of Mentha pulegium and histopathological evaluation of its ameliorating effect on experimental acute hepatic injury. Histol Cytol Embryol. 2017;1(1):1–5.
54. Lee CP, Shih PH, Hsu CL, Yen GC. Hepatoprotection of tea seed oil (Camellia oleifera Abel.) against CCl4-induced oxidative damage in rats. Food Chem Toxicol. 2007;45:888–895.
55. FahmyHwan NM, Al-Sayed E, Abdel-Daim MM, Karonen M, Singab AN. Protective effect of terminalia muelleri against carbon tetrachloride-induced hepato and nephro-toxicity in mice and characterization of its bioactive constituents. Pharm Biol. 2016;54:303–313.

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